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DOCKET NO.: UPAP0025-100 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: David B. Weiner *et al.*

Serial No.: 09/719,067 **Group No.:** 1632

Filing Date: August 16, 2001 **Examiner:** Ram Shukla

For: **METHODS AND COMPOSITIONS FOR DELIVERING PROTEINS TO MACROPHAGE CELLS AND CELLS OF MACROPHAGE DERIVED LINEAGES**

DECLARATION OF DR. DAVID B. WEINER PURSUANT TO 37 CFR § 1.132

I, Dr. David B. Weiner, declare as follows:

1. I am an inventor in the above-identified patent application.

2. Attached hereto are Exhibits 2-4 that indicate the level of skill and knowledge of those in the art at the time the above-identified application was filed. The exhibits demonstrate that at the time the invention was made, the drainage patterns associated with parts of the lymphatic system were reasonably well understood and reasonably predictable. The exhibits demonstrate that at the time the invention was made, one having ordinary skill in the art could determine the drainage pattern(s) of the lymphatic system and correlate a site of injection to a particular lymph node using routine methodology without undue experimentation.

3. Exhibit 2 states that "For ... the arms or legs, the lymphatic drainage patterns are fairly predictable: the arm drains to the axilla, and the leg drains to the groin." (Exhibit 2, Oncolog, taken from www3.mdanswerson.org/~oncolog/map.html, page 1). Exhibit 2 further states, "When the drainage patterns are ambiguous, lymphoscintigraphy is used to identify nodal basins...Lymphoscintigraphy begins with injection of a radiolabeled colloid into the

skin...Over the course of a few minutes, the colloid passes through the dermal lymphatics to one or more lymph node basins, where it is taken up by the macrophages in the lymph nodes. A scintillation camera is then used to document the path of the radiolabeled colloid through the lymphatic system." (Exhibit 2, page 2,).

4. Exhibit 3 describes in more detail the method of finding lymph nodes using lymphoscintigraphy. As described on page 1 of Exhibit 3 (taken from www.nucmednet.com/lymph.htm, entitled "Procedure of the Month November 1997), "since one of the functions of the lymph system is to clear small particles, the ...particles injected during lymphoscintigraphy go to the local lymph nodes. Since these particles have a radioactive label, we can find them using nuclear medicine equipment."

5. Exhibit 4 (Eddy et al. World J. Surg. (2001) 25:794-797) describes lymphoscintigraphy and lymphatic mapping for identification for sentinel lymph nodes. In describing the technique the authors sate "investigators obtain a lymphoscintigram to delineate lymphatic drainage." (Eddy, p. 796, left column).

6. These references demonstrate that at the time the invention was made one of skill in the art could identify the site of administration required to deliver a DNA molecule to a specific lymph node based upon the lymphatic drainage system that was either known or that was determined using techniques that would not require undue experimentation.

7. I declare that statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated:_____ By:_____

Dr. David B. Weiner

Exhibit 1: Curriculum vitae of Dr. David B. Weiner

Exhibit 2: Oncolog: Intraoperative Lymphatic Mapping—Elegant Way to Identify Lymph Node Metastases in Melanoma Patients taken from www3.madanderson.org/~oncolog/map.html

Exhibit 3: "NucMedNet Feature Procedure: New Techniques for Finding Lymphnodes in Cancer Patients" taken from www.nucmednet.com/lymph.htm.

Exhibit 4: Eddy et al. World J. Surg. (2001) 25:794-797

EXHIBIT 2 FOR DECLARATION OF

DR. DAVID B. WEINER

OncoLog

Intraoperative Lymphatic Mapping Elegant Way to Identify Lymph Node Metastases in Melanoma Patients

New procedure enables selective use of lymph node dissection

By Stephanie P. Deming

Like a number of tumors, cutaneous melanomas often spread through lymph channels to regional lymph nodes. Because of this tendency, elective lymph node dissection--removal of lymph nodes before there is clinical evidence of metastasis--has long been a standard treatment for patients with early stage cutaneous melanoma. While this procedure has not been proven to prolong survival, many surgeons believe that elective lymph node dissection in a patient with micrometastases can prolong the patient's life and in some cases cure the disease.

Until recently, however, this approach involved a catch-22: elective lymph node dissection could benefit nly those patients with micrometastases, but determining whether a patient had micrometastases required a lymph node dissection. Thus, some patients underwent unnecessary surgery--a matter of concern because lymph node dissection is a major surgical procedure associated with a number of potential short- and long-term complications.

A cutting-edge approach being studied in clinical trials at The University of Texas M. D. Anderson Cancer Center offers a way around this dilemma. Using this new technique--intraoperative lymphatic mapping and sentinel node biopsy--surgeons can determine the disease status of an entire lymph node basin by identifying, removing, and examining a single special lymph node called the sentinel node.

The sentinel node is the first node that the dermal lymphatics around a tumor drain to. Studies have shown that the pathologic status of the sentinel node accurately predicts the status of all of the lymph nodes along that drainage pathway. In other words, if the sentinel node is free of tumor, so are all of the other nodes, and formal lymph node dissection is not necessary.

"This procedure can help identify which patients are most likely to benefit from lymph node dissection and which patients probably would not benefit," said Merrick I. Ross, M.D., associate professor in the Department of Surgical Oncology. "It allows us to be more selective about performing lymphadenectomy."

Lymphoscintigraphy Reveals Nodes at Risk

Before performing intraoperative lymphatic mapping, the surgeon must know which nodal basins are at risk for micrometastases. For melanomas on the arms or legs, the lymphatic drainage patterns are fairly predictable: the arm drains to the axilla, and the leg drains to the groin. For lesions n the trunk, however, the drainage patterns are ambiguous. A melanoma on the upper trunk might drain to the groin, for example, or a melanoma near the left axilla might drain to the right axilla. And sometimes a lesion

drains to more than one nodal basin. "It's not uncommon to find two nodal basins," said Ross, "and it's not unheard f to find three. We will pursue these multiple nodal basins if necessary."

When the drainage patterns are ambiguous, lymphoscintigraphy is used to identify the nodal basins at risk. This simple outpatient procedure is typically performed several days before the intraoperative lymphatic mapping and sentinel node biopsy. Lymphoscintigraphy begins with injection of a radiolabeled colloid into the skin adjacent to the tumor. Over the course of a few minutes, the colloid passes through the dermal lymphatics to one or more lymph node basins, where it is taken up by the macrophages in the lymph nodes. A scintillation camera is then used to document the path of the radiolabeled colloid through the lymphatic system. This is the same path that tumor cells would follow if they broke away from the primary lesion and entered the lymphatics.

"Lymphoscintigraphy doesn't tell us if there's tumor in these areas," said Ross, "but it does tell us that if tumor had traveled to a lymph node area, that's where tumor would most likely be." With this information in hand, the surgeon can plan the intraoperative lymphatic mapping.

Dye, Radiolabeling Help Locate Sentinel Node

Intraoperative lymphatic mapping and sentinel node biopsy is performed at the same time as wide local excision of the primary tumor. The operation is typically performed as an outpatient procedure, with patients staying in the clinic about 23 hours.

About an hour before surgery, the patient is taken to the nuclear medicine station, where technicians inject a radiolabeled colloid into the skin adjacent to the tumor. The next stop is the operating room, where the surgeon injects a blue vegetable dye called isosulfan blue near the tumor.

While the blue dye travels through the lymphatic system, the surgeon scans the skin over the nodal basin with a hand-held, portable gamma probe, looking for areas with high levels of radioactivity. These "hot" areas signal lymph nodes that have taken up the radiolabeled colloid; the hottest area corresponds to the sentinel node.

The surgeon makes a small incision directly over the sentinel node and inserts the gamma probe, which is covered with a sterile sheath. By moving the probe around, the surgeon can further pinpoint the area of high radioactivity. Within this region, the surgeon hunts for a blue-stained node--the sentinel node--and carefully dissects it.

How does the surgeon know that the blue-stained node in question is actually the sentinel node? "There's a time element involved," said Ross. "If you wait too long, the dye can pass through several nodes. We generally do the biopsy within 20 minutes after injecting the blue dye." The lymphatic channels connecting the nodes are also stained blue, so "once you find the node, you can trace back the lymphatic channels leading to it to make sure it's the first node--that there isn't a node before that one."

When surgeons at M. D. Anderson Cancer Center first performed intraoperative lymphatic mapping, they relied on the blue dye alone to localize the node. The gamma probe was introduced about one and a half years ago. "When we were using just the dye," said Ross, "we were confident between 85 and 90 percent f the time that the node we found was the sentinel node, because we were limited to visual inspection. Since we've been using the gamma probe, we almost never have a concern about not finding the appropriate lymph node."

With use f the gamma probe, said Ross, "we know where the sentinel node is going to be. This allows

us to make a very small incision and also makes the operation much quicker."

After the sentinel node is removed, it is examined by a pathologist. If the node looks clinically suspicious, it is examined by frozen section. The results are available in a matter of minutes, and if the node contains metastases, the surgeon can proceed with a formal lymph node dissection in the same operative setting. However, "if the node looks normal," said Ross, "we prefer to evaluate the lymph nodes by serial sectioning with permanent sections. It is more accurate, and we are less likely to miss tumor. We are looking for a small amount of microscopic disease, and you can sometimes lose important tissue when you do a frozen section." In this case, if the sentinel node contains micrometastases, the lymph node dissection is performed at a later date.

New Procedure Offers Several Advantages

This new procedure offers a number of important advantages over traditional treatment, chief among them the ability to avoid formal lymph node dissection and its attendant risks--including obvious scarring, nerve damage, or lymphedema--in patients who would not benefit from the procedure.

The new procedure may also allow better detection of micrometastatic disease. "There are patients who are thought to be lymph node negative who eventually have a recurrence," said Ross. "We think that a number of these patients are actually lymph node positive, but we missed the micrometastases because we weren't able to look at every lymph node carefully enough." With a traditional lymph node dissection, detailed examination of all the nodes removed is not feasible--the time and expense involved are prohibitive. However, with only one or two nodes to focus on, said Ross, "it is more feasible to perform very careful examination by using serial sectioning and immunohistologic studies," and thus the chances of detecting micrometastases are greater. The sentinel nodes are also thought to be the nodes most likely to harbor micrometastatic disease, so focusing on those nodes is the best strategy for detecting micrometastases.

Early detection of disease spread to lymph nodes is especially important now that alpha-interferon has been identified as an effective adjuvant therapy for patients with lymph node spread of melanoma. The earlier micrometastases in regional lymph nodes are identified, the earlier patients can receive this therapy.

Procedure Also Useful for Other Types of Cancer

Encouraged by the success of intraoperative lymphatic mapping and sentinel node biopsy for the treatment of cutaneous lymphoma, surgeons are investigating the role of this procedure in treating breast and other types of cancer. "Right now, the standard of care for patients who undergo surgery for breast cancer is to include an axillary lymph node dissection, but now that we're seeing breast cancer earlier, a lot f these patients don't have lymph node involvement," said Ross. In a preliminary trial of sentinel node biopsy in breast cancer patients, M. D. Anderson surgeons found only one false-negative result in a series of 35 patients. According to Ross, some areas of the breast drain exclusively to the internal mammary chain--not the axilla, the traditional site of lymph node dissection in breast cancer patients. "For patients with tumors in those areas of the breast," he said, "sampling the axilla may be misleading. Using intraoperative lymphatic mapping with a gamma probe, it is possible to access the internal mammary nodes. That has been out of vogue for some period of time, but now that we're understanding lymphatic drainage better, it may be coming back into our staging evaluations of patients with breast cancer."

Intraoperative lymphatic mapping can also be used for melanomas of the vulva and for other skin

cancers that spread to lymph nodes--some of the adnexal tumors of the skin, Merkel cell tumors of the skin, and some of the more aggressive squamous cell cancers. "This technique," said Ross, "is applicable to essentially any solid tumor that has a predilection for lymph node metastases."

REFERRALS. Readers who would like more information may write Dr. Ross, Department of Surgical Oncology, Box 106, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, or call (713) 792-7217. To refer a patient, call the New Patient Referral Office at (800) 392-1611 or (713) 792-6161.

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EXHIBIT 3 FOR DECLARATION OF

DR. DAVID B. WEINER

NUCMEDNET FEATURE PROCEDURE

New Techniques for Finding Lymph Nodes in Cancer Patients

Figure 1. Lymphoscintigraphic study of the right arm. The colloidal material was injected around the large dark area at the lower portion of the image. The colloid moved upward to show a sentinel lymph node (closest to the injection site) and then to satellite lymph nodes (see text below) in the shoulder area. The sentinel node should be removed to see if it contains tumor.

Introduction

One of the questions that is important to answer in patients with certain types of cancer is whether the cancer has spread to the local lymph glands. The patient's future treatment, as well as his or her chances for cure, is in large part determined by whether or not the lymph glands are involved.

Lymph glands have a number of functions. Normally, they are designed to trap organisms such as bacteria and bring to bear the body's immune system to stop the infection. They may also have a role in fighting cancers. However, when the cancer overwhelms the lymph node, the value of the node to the patient disappears.

For purposes of treatment, cancers are "staged." The staging process involves measuring the size of the original tumor and assessing evidence of cancer in other body organs and whether or not there is tumor in local lymph nodes. The traditional method of determining whether or not the lymph glands are involved is to remove them surgically and examine them in the pathology laboratory. This approach requires more extensive surgery since lymph nodes can be in variable locations. This more extensive surgery leads to a longer recovery time. Recently, surgeons and nuclear medicine physicians have been collaborating on a new method to allow the surgeon to find the key lymph nodes in the region. It is hoped that this method will permit less extensive surgery and accurately reflect the degree to which the cancer has spread at onset of treatment.

Lymphoscintigraphy

The technique, known as lymphoscintigraphy, involves injecting small amounts of radioactive particles (a colloid) around the tumor site. Since one f the functions of the lymph system is to clear small particles, the colloidal particles injected during lymphoscintigraphy go to the local lymph nodes. Since these particles have a radioactive label, we can find them using nuclear medicine equipment.

The key lymph node to identify is known as the "sentinel" node. This lymph node represents the first node in the drainage path from the tumor to the remainder of the body. Data from multiple hospitals indicate that if this lymph node is free of tumor, it is likely that other lymph nodes, further away, will not contain tumor. For the patient, this means if we can identify the sentinel node, a less extensive surgical procedure can be performed to sample this particular node.

There are several diseases in which the status of the lymph nodes is of critical importance to therapy. These diseases are malignant melanoma (a form of skin cancer), breast cancer, and colon cancer. In each of these diseases there are techniques that permit both the imaging and surgical evaluation of specific lymph nodes. In lymphoscintigraphy studies tracer is injected in a circle surrounding the tumor or the site from which the tumor has been removed. Images over the next several hours show the path of flow. In the past, when lymphoscintigraphy was performed, the skin over the lymph node, as seen on the images, was marked with waterproof ink. This presented problems for the surgeon since the position of the patient on the operating table may have been different than in the nuclear medicine department and, therefore, the skin marker may no longer be over the node.Today, special gamma probes exist that can be sterilized for use in the operating room. The surgeon can pass this probe over the area of the suspected lymph node and watch a gauge. When the gauge begins to show radioactivity, the probe is over the site of the sentinel lymph node. We will review some of the specific applications of lymphoscintigraphy below.

Malignant Melanoma

Malignant melanomas of the back or chest present problems with regard to identification of where the lymph nodes draining this region are located. (Figure 1) Depending on the tumor site, one may guess that lymph glands draining the tumor may be under the arm or in the groin. Without actually studying the patient, it is not possible to be sure which set of lymph nodes needs to be sampled or removed. Previously, the surgeon used anatomic criteria to decide which nodes to remove. In some patients, the tracer flows to lymph nodes in the axilla. In other patients it flows to those located in the groin. At times the flow is to both axillary regions or to the groin and axilla. Since the surgeon has an excellent idea of where to locate the lymph nodes for sampling from the lymphoscintigraphy study, there is increased accuracy of nodal identification.

Figure 2. The colloid was injected around this centrally located tumor on the patient's back (dark area). Notice the tendrils of radiocolloid extending to both the right and left of the injection site. Both the right and left axillary (underarm) regions have lymph nodes draining from this tumor. The surgeon must sample both regions to be sure of the patient's stage.

Particularly in malignant melanoma, the status of the sentinel node is of key importance. When this node does not contain tumor, it is likely that other lymph nodes in the region will not contain tumor. Therefore, in many centers it is now the practice to sample the sentinel lymph node and perform further surgery only if tumor is identified in this node. From the patient's perspective, this can result in considerably less surgery if the sentinel node is normal. When there is a question concerning whether or

not additional lymph nodes need to be removed, the decision can be based on hard data, such as the sentinel node's findings, rather than on opinion.

Breast Cancer

Breast cancer tends to spread to the lymph nodes under the arm (axilla) or behind the breastbone (retrosternal). Sampling of lymph nodes under the arm is a routine surgical procedure in breast cancer patients. However, there is no guarantee that the correct lymph nodes are always being sampled. Lymph flow is very variable and any one of a number of lymph nodes may represent the sentinel node. By using a lymphoscintigraphy study as a guide, the surgeon can understand where the sentinel node is located prior to the operation. Using a probe at the time of surgery, the precise location of this node can be determined for removal.

When the disease spreads to the retrosternal nodes, they generally are not surgically removed. Instead, radiation therapy is employed to treat these areas. While in some situations retrosternal nodes will be treated whether or not there is evidence of disease, it makes more sense to treat those in which the probability of disease is very high. Lymphoscintigraphy, by being able to identify potentially involved lymph nodes, allows therapy to be directed to the appropriate area.

Colon Cancer

Colon cancer spreads through the abdomen via the lymph channels. At the time of surgery the highest probability of cure is attained if the draining regional lymph nodes are removed. However, the colloids used for other lymphoscintigraphy studies are not practical for use in this setting. Radiolabeled antibodies are employed in this setting. These antibodies are directed against portions of the tumor surface that contain unusual proteins. By locating the sites of abnormal protein concentration, the study assists the surgeon in identifying areas likely to contain tumor. When radiolabeled antibodies are employed to locate tumors at the time of surgery, the technique is known as radioimmunoguided surgery (RIGS).

Patient Preparation

There is no specific preparation required for this examination. It is desirable to perform the lymphoscintigraphy study prior to tumor surgery. This is not always possible since the tumor may have been removed to make the diagnosis. The entire imaging procedure can take from one to four hours depending upon the speed with which the injected drug moves through the lymph system. In many patients the sentinel lymph node is identified within the first hour after injection. In other patients it may take several hours to identify the appropriate node.

There is mild discomfort associated with the injections. As noted above, several injections in the skin made in a circle surrounding the area the tumor previously occupied. There may be a slight burning sensation at the time of injection. The radiation dose is quite low since only a small amount of radioactivity is used for the injection. There are no reactions to the drug and no long-term consequences from the injections. When radiolabeled antibodies are used, it is typical to inject a week or more prior to surgery. These antibodies have a small chance of producing an allergic reaction. Patients with allergies to multiple substances should inform the physician f their history of allergies. While drug reactions are rare, they can occur and are usually easily handled if the physician is aware f the possibility.

Outcomes

Various published papers indicate that in patients with malignant melanoma, about 20% of the sentinel nodes actually contained tumor. For the remaining patients, extensive surgery was not appropriate. In a group of breast cancer patients, 92% of the sentinel nodes were identified. The sentinel node contained tumor in a small number of patients and was the only positive lymph node. When the sentinel node was negative no other lymph nodes contained tumor.

Further Information

For specific information on how the nuclear medicine department in your area performs this test, we suggest you contact them. They can also inform you if any special preparation is required.

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EXHIBIT 4 FOR DECLARATION OF

DR. DAVID B. WEINER

#3

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Lymphoscintigraphy and Lymphatic Mapping for Identification of Sentinel Lymph Nodes

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Abstract. The recently introduced technique of sentinel lymph node dissection (SLND) may replace complete axillary lymph node dissection for axillary staging of early breast cancer. Successful SLND is predicated on meticulous delineation of the lymphatic pathway and sentinel node(s). Currently employed lymphatic mapping materials include vital blue dyes and radioactive tracers. Techniques of intraoperative lymphatic mapping and SLND using dye, tracer, or both have high success rates in the hands of experienced investigators, but their routine and widespread use awaits resolution of questions about the timing, dose, and type of radioactive tracer; the optimal lymphatic mapping technique; indications and contraindications for SLND; and certification of qualified surgeons, pathologists, and nuclear medicine physicians.

Attempts to map the lymphatic system began more than 370 years ago. In 1622 Asellius described the lacteals in the mesentery of well-fed dogs [1]. Subsequently, the cisterna chyli and thoracic duct were described by Pecquet (1651), Bartholin (1653), and Rudbeck (1653) [1]. The first description of the lymphatics of the breast appeared more than 100 years later in Cruikshank's 1786 report detailing the two main drainage pathways of the breast: the axillary route and the internal mammary route [2]. Almost a century later (1875) Sappey's exhaustive delineation demonstrated the extensiveness of the lymphatic system [2]. During the 1930s investigators observed that any penetration underneath the epithelium opens small, normally unused lymphatic vessels, so an intradermal or parenchymal injection is, in effect, an intralymphatic injection [3–5]. This observation subsequently led to intraoperative delineation of the lymphatic drainage basin receiving dyes that had been injected into or around a tumor. However, because the lymphatic pathway to this drainage basin was often within the surgical field, these seminal studies did not affect surgical management.

During the late 1950s, investigations of the lymphatic system leaped forward with the introduction of lymphangiography using radiopaque contrast material to visualize lymphatic channels in vivo. In 1977 Cabanas [6] described the use of lymphangiography to identify the first ("sentinel") lymph node receiving lymphatic drainage from a primary penile cancer, and he devised a blind biopsy technique to sample this fixed-location lymph node. However, because the lymphatic drainage patterns from other anatomic sites are not simple or consistent, Cabanas' sentinel node biopsy technique had limited application, and its significance was not fully appreciated. Broader application of lymphatic mapping from a primary tumor was made possible by the concurrent but independent development of radioactive isotope imaging with labeled gold colloid [7, 8].

In 1992 a dye-directed visual technique of sentinel node biopsy was introduced by Morton et al. [9] for patients with primary cutaneous melanoma. This clinical study was based on their preclinical investigations showing that intradermal injection of a vital blue dye in an extremity consistently stained a sentinel lymph node in the regional draining basin [10]. The current clinical technique of intraoperative lymphatic mapping and sentinel lymph node dissection (SLND) for melanoma evolved from Morton's work, with the recent addition of immunohistochemical examination of the sentinel node specimen for micrometastases and refinements of the lymphoscintigraphic technique, including the introduction of technetium 99m-labeled sulfur colloid as the tracer material.

The success of SLND for melanoma suggested its potential for other solid tumors such as breast cancer, gastrointestinal cancer, head and neck cancer, and lung cancer, where nodal staging is also important. Several lymphatic mapping techniques have been used by various sentinel node investigators. These techniques employ a vital dye, a radioisotope, or both to identify the sentinel node. All are based on the principle that lymph from a primary tumor drains first to one or a few select lymph nodes in the lymphatic basin, but each is distinguished by technical variations. This paper focuses on the evolution and success rates of SLND techniques currently used for axillary staging of breast cancer.

Dye-Directed Lymphatic Mapping and SLND

To map the sentinel node in the axillary lymphatic drainage basin of patients with primary breast cancer, we adapted the dye-directed SLND technique introduced by Morton et al. [9] for map-

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ping dermal lymphatics in patients with melanoma. W used the same isosulfan blue dye (Lymphazurin) used in melanoma but adjusted its injection time, volume, and site to accommodate the mechanics of parenchymal lymphatics draining the breast. Our pilot study [11] included 174 women who had T0 to T3 breast cancer with or without a clinically positive axilla. All patients underwent complete axillary lymph node dissection (ALND) after SLND to confirm the sensitivity and accuracy of sentinel node mapping. Dye was injected in or around the tumor at various time intervals until an optimal technique was developed. The blue-stained lymphatic channel was identified and followed proximally to the lateral edge of the breast parenchyma. This study established that both the interval between dye injection and axillary dissection and the volume of dye injected (3–5 ml) depend on the distance between the primary tumor and the axillary basin. The study also set criteria for histopathologic examination of the SLND specimen. These criteria resulted from investigation of the five false-negative results. Because three of the five were misidentification of blue-stained fat as a sentinel node, the frozen section technique was introduced to verify the presence of nodal tissue. Because one of the remaining two false-negative results became positive when the SLND specimen was reexamined with immunohistochemical staining (IHC), IHC was introduced to evaluate sentinel nodes that were tumor-free on hematoxylin-eosin staining (HE).

Not surprisingly, our pilot study revealed a learning curve for successful mapping of the sentinel node: The SLND success rate of 59% for the first half of the study jumped to 72% for the second half and to 78% for the last 50 cases. This increase reflects the fact that the SLND technique itself was being developed as the study progressed, and no patient was excluded from study, even those in whom we injected an insufficient volume of dye at inappropriate time intervals. Our validation study of the mature SLND technique for breast cancer was undertaken in 107 patients with clinical T1-T2 breast cancer and clinically negative axillae [12]. Again, all patients underwent SLND followed by ALND. At least one blue-stained sentinel lymph node was identified in 94% of cases, and the histopathologic status of this node invariably matched that of other axillary nodes. The results of this validation study allowed us to abandon routine completion ALND in patients whose sentinel nodes had no evidence of tumor when examined with HE and IHC.

Our confidence in the mature dye-directed SLND technique is further bolstered by data gathered from an exhaustive histopathologic analysis of nonsentinel nodes in 60 patients [13]. We undertook IHC assessment of 1087 nonsentinel nodes removed from 60 breast cancer patients who had no IHC evidence of tumor cells in multiple sections of their sentinel nodes. These patients were identified from a cohort of 103 consecutive breast cancer patients undergoing SLND followed by ALND. Only one tumor-positive nonsentinel node was identified, an error rate of 0.1% (1/1087) and an axillary status staging error rate of 0.9% (1/103).

Our current dye-directed SLND technique begins with a subcutaneous injection of 3 to 5 ml of isosulfan blue dye into the peritumoral breast parenchyma on the axillary side. If biopsy was performed prior to SLND, dye is injected into the breast parenchyma adjacent to the biopsy cavity. Care is taken to avoid injecting dye into the underlying pectoralis fascia or skin. As stated above, the volume of the injectate depends on the distance of the primary tumor from the axilla. After dye injection and before axillary skin incision, gentle compression of the area for 5 to 7 minutes moves the dye along the lymphatics. The skin is incised just below the hair-bearing area in the axilla, and the incision is carried down to the axillary fat pad. It is not unusual to encounter cutaneous dye-filled lymphatics during this part of the procedure. They can be divided without compromising the outcome of SLND. As the axillary fat pad is entered, blunt dissection in the axilla is performed to identify a dye-filled lymphatic tract. Once the tract is identified, it is followed distally to the blue-stained lymph node and proximally to the lateral edge of the breast parenchyma to ensure that the blue-stained node is the first node to receive lymphatic drainage from the tumor (i.e., the sentinel node). If there is more than one dye-filled lymphatic tract, each is followed. Although it is not uncommon to find two or more sentinel nodes, multiple tracts often lead to the same lymph node.

Although we prefer isosulfan blue dye, other vital dyes have been used by other investigators with equally successful results. Koller et al. [14] used 1% methylene blue dye and patent blu V dye interchangeably for their study of SLND in 98 breast cancer patients. A sentinel node was identified in 96 patients (98%), and the false-negative rate was 6%. The rate of sentinel node identification was the same for both dyes. Flett et al. [15] used 2.5% patent blue dye to identify a sentinel node in 82% of 68 patients, and their false-negative rate was 14%. Both groups of investigators injected the dye into the breast parenchyma surrounding the tumor, and both groups made the axillary incision 5 to 10 minutes after dye injection.

The dye-directed mapping technique is simple in concept yet demanding in detail. The dye must be precisely injected in or around the tumor; the interval between injection and incision must be long enough to allow staining of the sentinel node; dissection must be meticulous and blunt; and the search for a sentinel node must be thorough. Preoperative lymphoscintigraphy should be considered for patients whose tumors are in the medial hemisphere of the breast to document axillary drainage. It also should be used for any patient who may have a nonaxillary drainage pathway, although the likelihood of isolated metastases to nonaxillary nodes is less than 10% [16, 17].

Probe-Directed Lymphatic Mapping and SLND

Probe-directed lymphatic mapping and SLND in breast cancer was first described by Krag et al. [18], who used about 0.4 mCi of unfiltered technetium 99m (^{99m}Tc)-labeled sulfur colloid (SC) as the radioactive tracer. Several independent investigators have verified the accuracy of probe-directed SLND [19–23]. Veronesi et al. [19] used about 7 MBq of ^{99m}Tc-labeled colloidal albumin in 0.2 ml saline; the day before surgery this tracer was injected subdermally above the breast lesion. Borgstein et al. [20] used 40 MBq of ^{99m}Tc-labeled colloidal albumin in 4 ml saline; tracer was injected in two to four spots around the breast lesion on the day before surgery. Miner et al. [21] used ultrasonography to guide the injection of 1 mCi of unfiltered ^{99m}Tc-SC (in 4 ml of saline) into four defined locations around the tumor. Surgery was undertaken 1 t 9 hours (median 3.5 hours) after injection. Offodile et al. [22] used 1 mCi of ^{99m}Tc-labeled dextran in a volume of 0.5 ml. Crossin et al. [23] used 1 mCi of ^{99m}Tc-labeled SC in 4 ml of saline, which was injected 1 to 4 hours before surgery. In a recent multicenter trial [24] surgeons from 11 centers in various practice settings used 1 mCi of ^{99m}Tc-SC in 4 ml; it was injected in divided

aliquots into four defined peritumoral locations. Despite the considerable interstudy variations in mapping agents and technique, these studies were successful because each group of investigators carefully defined their respective SLND protocol and were consistent in its application.

In general, radiopharmaceuticals in the form of ^{99m}Tc-labeled unfiltered SC, filtered SC, human serum albumin colloid, or dextran are injected into the peritumoral parenchyma or subcutaneous tissue. Most but not all investigators then obtain a lymphoscintigram to delineate the lymphatic drainage and identify any "hot spot" (sentinel node) in the draining basin. The hot spot can be marked by the nuclear medicine physician to facilitate axillary incision for SLND. About 2 to 24 hours after tracer injection the patient is taken to the operating room and a small incision is made over the area of maximal radioactivity. Prior to skin incision, background radioactivity is measured over the sentinel node basin (preincision radioactivity count) and over a neutral site (background count). After skin incision, the gamma probe is used to guide dissection through each layer of tissue to a radioactive sentinel node. Occasionally, more than one node is radioactive, in which case each is designated a sentinel node. The radioactivity of the nodal basin is then measured after excision of the sentinel node. If the residual basin radioactivity is high, further excision is performed to remove any node containing high radioactive counts so the residual basin count is not significantly different from the background count.

Probe-directed SLND is highly sensitive for detecting nonaxillary drainage pathways. In a multiinstitutional study by Krag et al. [24], 11% of hot spots were outside level I of the axilla and 8% were outside the axilla. Thus probe-directed SLND may be especially useful in patients whose tumors are in the medial hemisphere of the breast. However, if the primary breast tumor is close to the axillary drainage basin, a shine-through effect may mask detection of a hot spot in the axilla. In the same study from Krag's group [24] all of the unsuccessful SLND procedures occurred in patients whose tumors were in the outer half of the breast.

Combined-Agent Mapping and SLND

Several SLND investigators have combined dye-directed and probe-directed mapping techniques to achieve success rates higher than 90% [25–28]. Albertini et al. [25] injected 16 MBq of ^{99m}Tc-labeled filtered SC 2 to 4 hours before surgery, followed by isosulfan blue dye 10 to 15 minutes before surgery. They reported no false-negative results among 57 patients who had successful SLNDs. Barnwell et al. [26] injected 3 ml of a mixture of 1% isosulfan blue dye and 1 mCi ^{99m}Tc-labeled SC 60 to 90 minutes before surgery. They also reported no false negatives among 38 patients. O'Hea et al. [27] injected 0.3 mCi of ^{99m}Tc-labeled unfiltered SC into the breast parenchyma adjacent to the tumor 2 to 4 hours before the surgery and then 4 ml of 1% isosulfan blue dye 5 to 10 minutes before axillary incision. Their false-negative rate was 15%.

In certain cases, a combined-agent approach may be optimal. For example, if the axillary drainage mapped on the lymphoscintigram is faint or if the search for a blue-stained axillary node is likely to be difficult, we would combine dye-directed and probe-directed intraoperative mapping techniques.

Discussion

The sentinel node hypothesis states that the histopathologic status of the first node on the lymphatic drainage pathway from a primary tumor reflects the tumor status of the entire lymphatic drainage basin. Underlying this hypothesis is the assumption that the surgeon can correctly and consistently identify this node (i.e., the sentinel node). It requires practice because the sentinel node can be difficult to identify regardless of the lymphatic mapping technique. Moreover, if a patient has metastasis only to the sentinel node, as occurred in 27% to 67% of cases reported in the literature [11, 12, 14, 18–21, 25–27], mistaking a secondary node for the sentinel node would result in understaging. Ironically, incorrect SLND introduces a larger error than does unsuccessful SLND because incorrect identification of the sentinel node can lead to understaging and inadequate treatment, whereas unsuccessful SLND leads to completion ALND and appropriate nonsurgical adjuvant therapy.

The most important factor when deciding whether SLND can replace ALND as a staging technique for breast cancer is its false-negative rate. The multicenter study by Krag et al.[24] revealed a considerable range of false-negative rates among participating surgeons, confirming that the surgeon's skill and experience are essential . Although we prefer dye-directed SLND and have performed this technique with almost 100% success in recent cases [29], others have used probe-directed or combined-agent SLND with equally impressive results. In each case the investigators defined the technical details related to their protocol for SLND and were consistent in their approach. Successful SLND depends on the technical maturity of the operating surgeon, the pathologists, and the nuclear medicine physician. All surgeons undertaking SLND for patients with primary breast cancer must identify their false-negative and success rates using a uniform technique. In addition, successful use of SLND depends on meticulous specimen analysis by the pathologist and the cooperation of the nuclear medicine physician. Finally, it requires detailed discussion with the patient regarding the possible outcome from the procedure and the experimental nature of this technique. Underway are two multicenter phase III trials of SLND for patients with breast cancer. One is sponsored by the American College of Surgeons Oncology Group and the other by the National Surgical Adjuvant Breast and Bowel Project. The results of these two studies will likely define the role of SLND in the management of breast cancer.

Résumé

L'introduction récente de la technique de biopsie du ganglion lymphatique «sentinelle» (BGLS) pourrait remplacer le curage complet de l'aisselle pour le staging du cancer du sein à son début. Le succès de la BGLS dépend de la délinéation méticuleuse des voies lymphatiques et de(s) ganglion(s) lymphatique(s) «sentinelle(s)». A présent, la cartographie lymphatique peut être obtenue par des colorants bleus vitaux et des isotopes radioactifs. Le taux de succès des techniques de cartographie peropératoire et de BGLS utilisant des colorants et/ou des traceurs radioactifs est élevé dans les mains des investigateurs expérimentés, mais la méthode doit être confirmée avant de préconiser leur utilisation systématique. Notamment on doit connaître les réponses aux questions en ce qui concerne le bon moment, la bonne dose et

type de traceur radioactif, la technique de cart graphie optimale, les indications et les contra-indications de la BGLS ainsi que la certification des chirurgiens, des anatomo-pathologistes et des médecins nucléaires pour cette technique.

Resumen

Las técnicas de disección del ganglio centinela (DGC) recientemente planteadas pueden reemplazar la disección axilar completa en la estadificación del cáncer mamario en sus etapas tempranas. Se logra una DGC exitosa mediante meticulosa demostración de las vías del drenaje linfático y del ganglio o ganglios centinela. Los materiales de mapeo linfático en boga en la actualidad incluyen los colorantes azules vitales y los trazadores radioactivos. Los métodos de mapeo intraoperatorio y de DGC que utilizan colorante y/o trazador exhiben una elevada tasa de éxito en manos expertas, pero su uso rutinario y amplio todavía espera la resolución de interrogantes relativos a la oportunidad en el tiempo, la dosis y el tipo de trazador radioactivo, la técnica óptima de mapeo, las indicaciones y contraindicaciones para DGC, así como la certificación de idoneidad de cirujanos, patólogos y especialistas en medicina nuclear.

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